REMARKS

Claims 18-27 and 29-35 are currently active.

Claim 28 has been canceled.

The applicants have amended the specification to more specifically claim priority from the original filing date of September 23, 1994.

The Examiner has rejected Claims 18-32 and 35 as being anticipated by Meltzer.

Applicants respectfully traverse this rejection. Meltzer fails to teach or suggest a centrifugation and supernatant removal procedure according to Claim 18.

It is noted that the Examiner in regard to Meltzer, on page 8 of the Office Action, states that Meltzer places the separated piece into a container, centrifuging to make a pellet and withdrawing supernatant to obtain DNA (page 4976, column 2, paragraph 3) by teaching that the separated tissue was placed into a container, ground into a powder, incubated in a container in a lysis solution, treated with phenol/chloroform and ethanol which involved centrifugation to make a pellet and removal of supernatant containing DNA. Applicants take

this opportunity to elaborate on Meltzer to explain why the limitations of the centrifugation and supernatant removal steps of Claim 18 are not taught by Meltzer.

The article by Meltzer et al (Proc Natl Acad Sci USA [1991] 88: 4976-4980) differs sharply and fundamentally with respect to the claimed invention. While the article in both abstract and discussion speaks about methods for genetic analysis that would be more applicable for clinical application in fact the actual methodology used possesses serious drawbacks. These drawbacks are precisely addressed by the claimed invention which provides the necessary solutions to enable effective clinical application. These drawbacks are:

1) Meltzer et al. require DNA isolation by phenol chloroform extraction. Fixative treated tissue undergo extraction with very low efficiency, less than 5%, making it necessary to sacrifice a relatively large amount of tissue to get sufficient material to do genetic analysis. This is a most serious drawback and would not permit clinical application of the methodology described in the report. The claimed invention totally circumvents this step by its ability to facilitate amplification enabling even small specimens such as biopsies to prove adequate for genetic analysis.

2) Meltzer et al. state that tissue sections were sectioned at 10 microns thickness. This thickness is 2.5 times thicker than routine sections are cut. Also, they do not state how many serial sections were used but it is implied that they used many serial sections to get

enough material for phenol/chloroform phase separation. They specifically do not state that a single tissue section is sufficient for analysis. Their approach would invariably result in the consuming of precious tissue specimens in order to provide adequate material for extraction (see #1 comment here). Serious sacrifice of the biopsy tissue block would not be tolerated in clinical practice whereas in contrast the claimed invention provides the necessary procedure to avoid undue loss of tissue. Moreover, all this additional material will create noise that will cause identification of the disease of the tissue to be extremely difficult.

3) Meltzer et al. describe centrifugation but this is in the context of centrifugation for phenol/chloroform phase separation, not for the creation of a supernatant to obtain DNA sequences from the target. There is no teaching or suggestion of applicants' withdrawing step in Meltzer et al. The claimed invention skips this highly unproductive step of phase separation for a more direct modified approach which is highly efficient. The centrifugation step in the claimed invention has no correlation with the centrifugation step of Meltzer et al.

4) Meltzer et al. require the use of washing steps with alcohol and reprecipitation of DNA which are inefficient steps and risk loss of vital small quantities of nucleic acid. The claimed invention uses a direct approach for tissue handling which avoid unwarranted loss of precious materials.

Like several articles written at that time and subsequently, the authors take liberty in describing their methods as enabling clinical application. By the same token, authors such as Meltzer et al liberally extrapolate their methods for easy clinical application without rigorously evaluating them for that purpose. The techniques described by Meltzer et al shares with the claimed invention the overall goal of reliable, simple, rapid and low cost genetic analysis of solid tissue specimens. Only the claimed invention is capable of fully delivering on that mission. This is accomplished by specifically avoiding tedious, expensive low yield extraction procedures in favor of optimized direct methodology to harmonize tissue pathology and genetic analysis.

Moreover, it was not intuitively obvious that centrifuging and separating the sample – with the consequent risk of loss of some DNA material to the discarded centrifuge pellet – would improve the known procedure for preparing the DNA material in a form and medium suitable for the amplification method.

This is especially true in light of the fact that a very small sample size is being used with the expectation that not enough material would be present to reveal critical information. On the contrary, the typical expectation would be to obtain large amounts of material to make sure enough information would be obtained to yield the critical information. In fact, the more material taken, the more difficult it is to obtain the critical information since

additional information from the additional material creates noise in the data that clouds or hides critical information.

The innovation of the claimed invention lies in the ability to amplify DNA from a minute quantity of tissue removed from precise topographic sites in histologic specimens. This enables the user to performed detailed molecular analyses from minute tissue specimens as well as from minute sites within larger sized tissue specimens. As such, it is of enormous value when examining clinical tissue specimens for the purpose of diagnosis and treatment planning.

Centrifugation is essential to the claimed invention and is very simple and fast, serving to separate cellular debris from vital constituents such as DNA that have been released by the preceding lysis step. Without centrifugation, subsequent DNA analysis will fail due to the interference related to cellular debris on succeeding steps. In other words, the centrifugation removes the "noise" from the cellular debris that would otherwise result in the analysis step if the debris was present. This step allows for a small sample size to be used and still yield critical information

The claims of the present application are, therefore, inventive over the prior art.

The Examiner has rejected Claim 33 as being unpatentable over Meltzer in view of Erlich. Erlich adds nothing to Meltzer, in relevant part to arrive at, applicants' claimed invention.

The Examiner has rejected Claim 34 as being unpatentable over Meltzer in view of Erlich and Teramoto. Teramoto adds nothing to the teachings of Meltzer in relevant part to arrive at applicants' claimed invention.

In view of the foregoing amendments and remarks, it is respectfully requested that the outstanding rejections and objections to this application be reconsidered and withdrawn, and Claims 18-27 and 29-35, now in this application be allowed.

CERTIFICATE OF MAILING

I hereby certify that the correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on 4/6/04

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